Effect of SSF Parameters for Corn Fiber

Tammy Kay Hayward
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AMOCO CRADA Bench Scale Research Director: Christos Hatzis

Objectives:

To study the effect of 5 SSF parameters for corn fiber. The experiment was designed as a fractional factorial in order to rank the main effects and study interactions. The design used was a half fraction 2^5 with 2 duplicate center points. The five factors were: corn fiber concentration, cellulase enzyme loading, glucoamylase loading, corn steep liquor concentration and SSF temperature.

Materials and Methods:

: The substrate used in this experiment was the corn fiber sent to NREL by AMOCO in December of 1994. Material from bucket #11 was stirred and then neutralized with lime to pH 5. The was then autoclaved in each flask prior to SSF initiation. The bucket #11 slurry was considered to be 100% . For a 40% w/w concentration in the flask. 40 grams of would be in a flask with a final SSF weight of 100 grams.

CSL: The nutrient source employed was 1% v/v Grain Products Corporation Corn Steep Liquor. This CSL is a very thick mixture containing solids. So, a 10% dilution of the CSL in DI water was adjusted to pH 5 with ammonium hydroxide, and autoclaved for 30 minutes. This autoclaved stock solution was then filter sterilized and added to autoclaved SSF flasks.

Cellulase: The PDU lot of CPN was used as the cellulase enzyme. A 10x dilution in D.I. water was filter sterilized and employed. The activity of the filtered, undiluted enzyme, as measured by Bill Adney was 70 FPU per mL. The enzyme solution also contains 300 g/L sucrose which had to be accounted for in the ethanol yields.

Amylase: Sigma glucoamylase from A. niger was used in these experiments. A 10x dilution was prepared in DI water and filter sterilized. The activity of the filtered, undiluted enzyme as stated on the bottle is 6100 units per mL. The enzyme is suspended in one molar glucose which also has ramifications in the ethanol yield.

Yeast: The organism used in this experiment was supplied by Ray Bigelis of AMOCO in December of 1994. A freeze back of this culture was performed. The vials were stored in the new -75 C freezer. The first stage inoculum was grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) prepared from a vial of the parent strain Labatt 1400 and grown for 12 hours at 38°C. The second stage was grown on 2% CSL and 2% Glucose at 38°C for 12 hours. At that time, the glucose in the flask was still above 10 g/L. Based on discussions with Nancy Combs, I placed the inoculum flask in a 30°C shaker and incubated for another 4 hours. After that time, glucose dropped to 3.5 g/L. This particular freeze back of Labatt 1400 seemed to perfer 30 over 38°C. A 10% v/v inoculum was then used to start the SSFs.

Conditions: SSFs were run in 250-mL flasks with 100 grams total working weights at 150 rpm with bubble traps. The ECF slurry was autoclaved in the flasks for 30 minutes at 121°C.

Experimental Design:
The run order of the flasks was randomized. Each flask had its own recipe. 18 total flasks were run at the same time, in three separate shakers, leading to a mix up on the amylase addition to the flasks. Appendix 1 depicts the actual run order and condition for each SSF flask.

Table 1: Conditions Tested

Factor	+/- Level Tested
Concentration of Corn Fiber	80 and 40% w/w
Cellulase Loading	15 and 5 FPU/g cellulose
Glucoamylase Loading	200 and 0 units/g souble six carbon sugars
Concentration of Corn Steep Liquor	1 and 0 % v/v
SSF Temperature	38 and 30°C

The conditions for the duplicate center points were 60% 10 FPU cellulase, 100 units amylase, 0.5% CSL at 34°C.

Results:
Table 2 shows the effect of each main factor on the three basic responses monitored. Ethanol yield responses are in % of theoretical yield. Residual glucose response is in grams per liter.

Factors	2 day EtOH yield	5 day EtOH yield	Residual Glucose
Corn Fiber	-7.21	-5.65	1.31
Cellulase	17.78	14.55	-0.07
Glucoamylase	4.78	6.79	-0.24
CSL	-1.46	0.77	-0.13
Temperature	3.22	-0.87	0.94

Effect of Corn Fiber Concentration: The higher level of corn fiber (80%) showed a significant negative effect on ethanol production level at 2 days. However, when ethanol production at 5 days of SSF is used, there is less difference in ethanol yields between 40 and 80% extruded corn fiber. The increased concentration of pretreated material seems to have more of an effect on the initial rate of ethanol production as compared to the final yield. This may be due to the increase in pretreatment inhibitor concentration (acetic acid, furfural, HMF). Also the 80% flasks looked significantly darker after autoclaving, which might suggest the formation of maillard products which may also inhibit the fermentation. Finally, the thicker 80% solution may cause lower yields due to mixing and mass transfer problems.

Effect of Cellulase Loading: Increasing the cellulase loading from 5 to 15 FPU per gram of cellulose had a significant positive effect on ethanol yield. The standardized effects at 2 and 5 days respectively were 17.78 and 14.55. Of the effects studied, this one is the main effect.

Effect of Amylase Loading: Unfortunately the effect of glucoamylase is confounded due to an error in the addition of the enzyme to the flasks leading to an imbalanced design. Nonetheless, the data suggests a positive effect on ethanol yields. The standardized effects of increasing the loading from 0 to 200 units, at 2 and 5 days respectively were 4.78 and 6.79. Also the negative effect of

corn fiber is more pronounced with high amylase. The addition of amylase also lowered the concentration of oligomeric sugars present at the end of the SSF. The highest amylase loading tested, 200 units per gram of original six carbon oligomer, produced the most reduction of oligomers. See Figure 1 Bar Graph of Oligomeric Sugars.

Effect of CSL concentration: The addition of 1% Corn Steep Liquor to the SSF did not have an effect on ethanol yields at either the 2 or 5 day time points. Those flasks with no CSL produced just as much ethanol as their counterparts with 1%. The standardized effects were -1.46 and 0.77. It seems that the corn fiber itself contains enough nutrients to sustain the Labatt 1400 yeast in batch mode.

Effect of SSF Temperature: Previous experiments with alpha-cellulose and pretreated hardwoods have shown an improvement in enzyme activity and subsequent ethanol yields with increasing temperature up to 40°C. Caution should be taken it that now we are talking about a different cellulase enzyme preparation and a different yeast. Increasing the temperature from 30°c to 38°C had standardized effects at 2 and 5 days of 3.22 and -0.87. These numbers are not considered significant. However, if the residual monomeric glucose at the end of SSF is used to examine the parameters an important discovery is made. Glucose build ups were found in SSF flasks at 38°C with 80% flasks and amylase. See Figure 2 Residual Glucose Cube Plot and Figure 3 Interaction of Concentration and Temperature.

Interactions: As mentioned above, glucose build up is an interaction between corn fiber concentration, temperature and amylase. The negative effect on ethanol yield of corn fiber is more pronounced at high levels of amylase. The standardized effect for this is -2.10. The negative effect of amylase and temperature is -1.53. All other two factor interactions are less than 1.5 at the 2 day time point.

Conclusions:

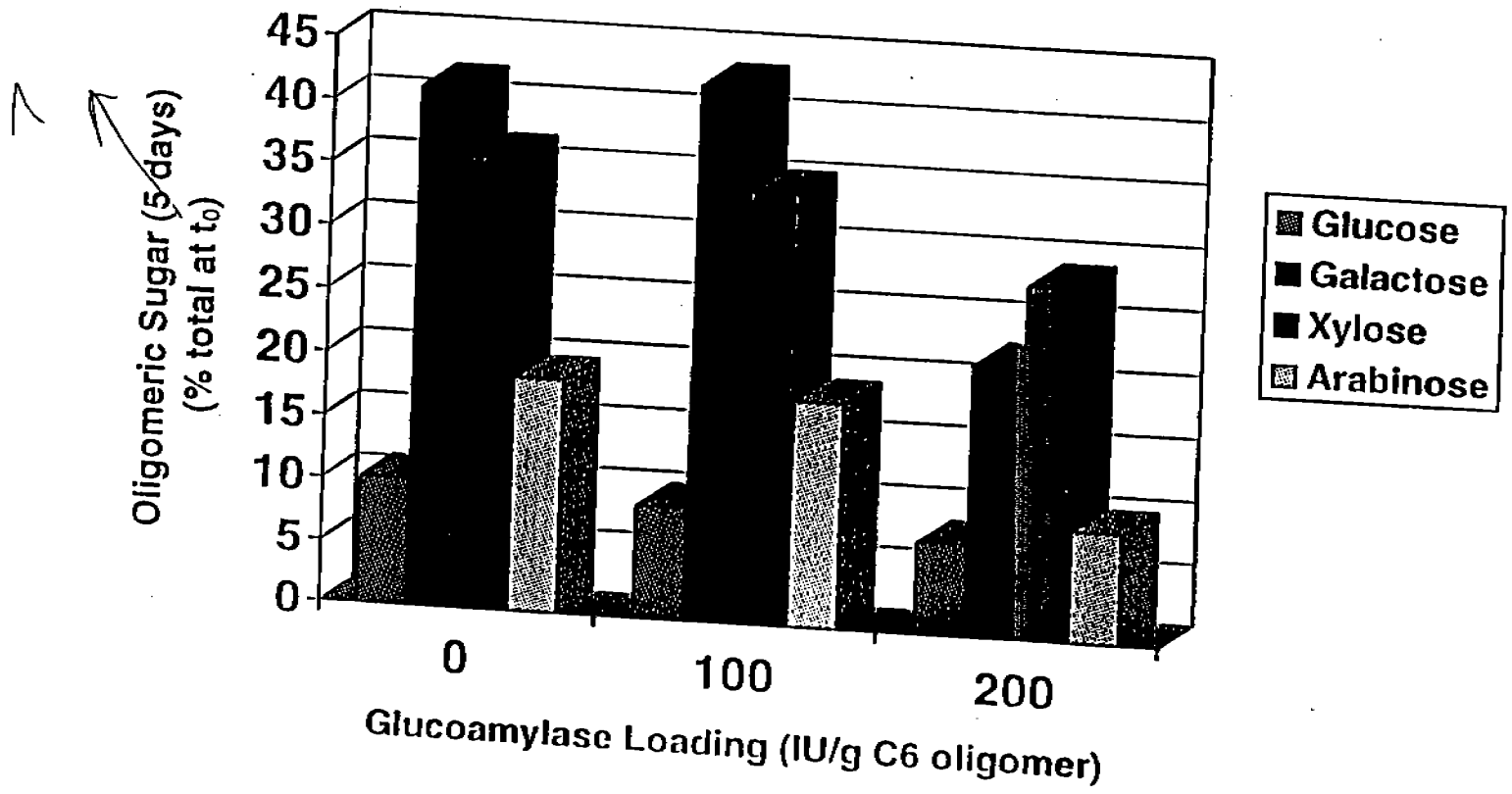
The ranked effects at the 5 day time point based on ethanol yield are Cellulase (14.54), Amylase (6.79), Corn Fiber (-5.65), Temperature (-0.87) and CSL (0.77). The main factor in ethanol yield of corn fiber SSFs with Labatt 1400 parent strain yeast is cellulase loading in the tested of range of 5 to 15 FPU. The SSF will build up glucose in 80% corn fiber with amylase at 38°C. Batch experiments do not seem to benefit from the addition of 1% CSL. Suggested standard batch SSF conditions with this substrate and yeast are no CSL, 34°C, 10 FPU/g cellulose, 200 units of amylase/g C6 oligomer, 40% corn fiber. Although an economic analysis has not been performed to determine the optimal amount, 15 FPU is not suggested based on the high cost of cellulase enzyme.

Figure 1: Bar Graph of Oligomeric Sugars
Figure 2: Cube Plot of Residual Glucose
Figure 3: Interaction of Corn Fiber Concentration and Temperature
Figure 4: Cube Plot of 2 day Ethanol Yields
Figure 5: Cube Plot of 5 day Ethanol Yields

Appendix 1: Standarized Effects
Appendix 2: CAT Task Analysis of selected Liquors
Appendix 3: Experimental Design

Figure 1

Effect of Glucoamylase on Oligomers



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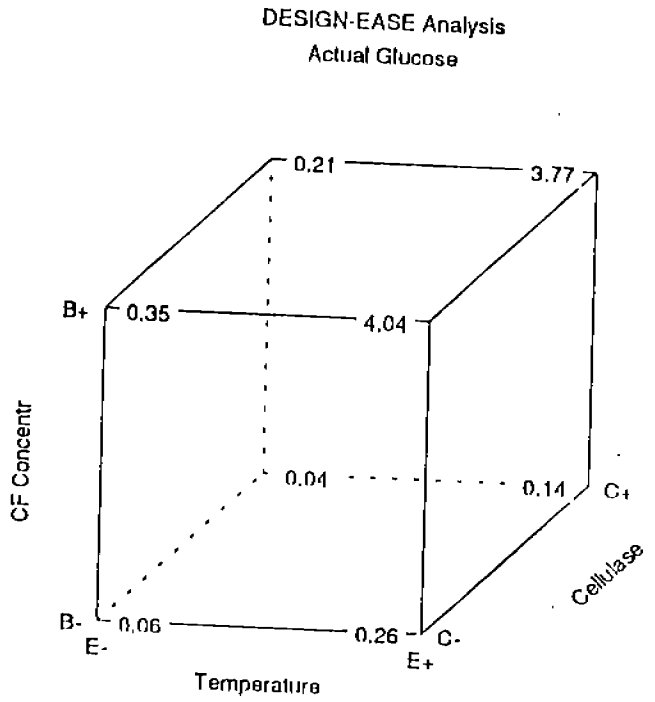
Residual Glucose



- Corn fiber concentration has a positive effect, indicating possible inhibition
- Temperature also has a positive effect
- There is a significant positive interaction between temperature and corn fiber concentration (temperature has no effect at low CF concentration and vice versa)
- Glucoamylase concentration has a significant positive effect

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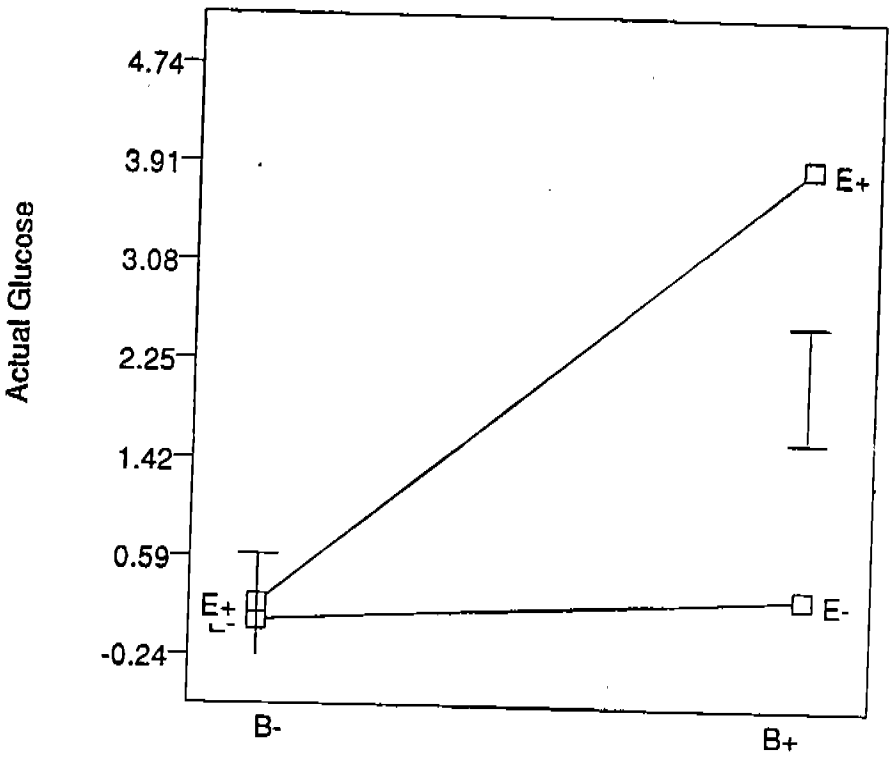
Figure 3

DESIGN-EASE Analysis

Glucose

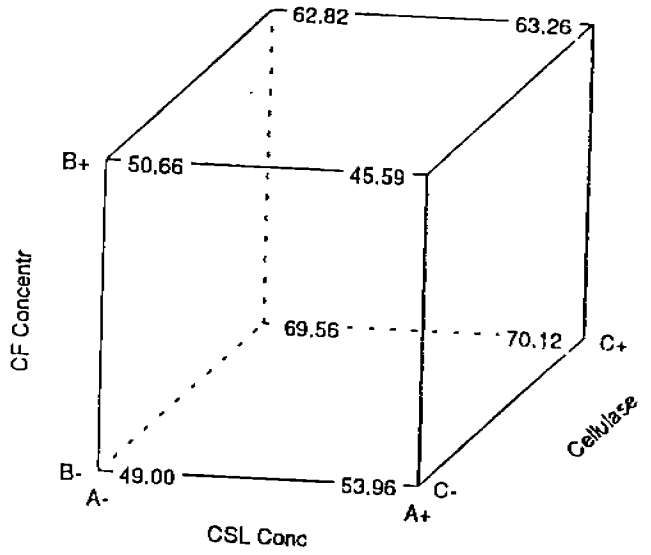


Interaction of B:CF Concentr and E:Temperature

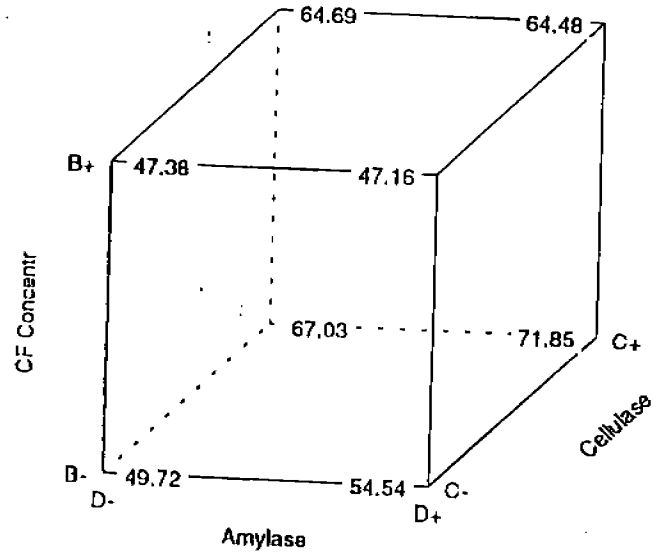
Ethanol Production (2 days)

Effects on Ethanol Yield

DESIGN-EASE Analysis
Actual Ethanol (2d)



DESIGN-EASE Analysis
Predicted Ethanol (2d)



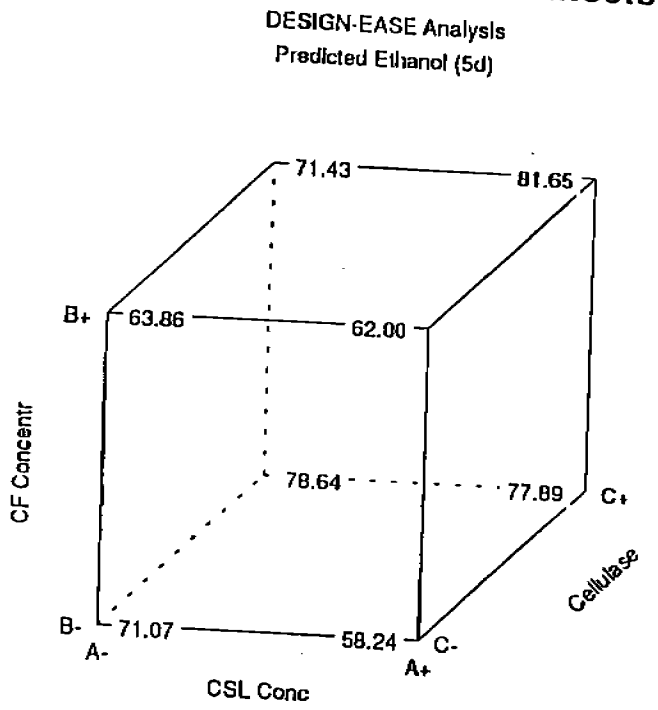
- CSL addition does not have an effect
- Corn fiber concentration has a significant negative effect
- Cellulase concentration has a very strong positive effect
- Glucoamylase concentration has a significant positive effect
- There is a significant negative interaction between corn fiber and amylase loading (the negative effect of CF concentration is more pronounced with high amylase)

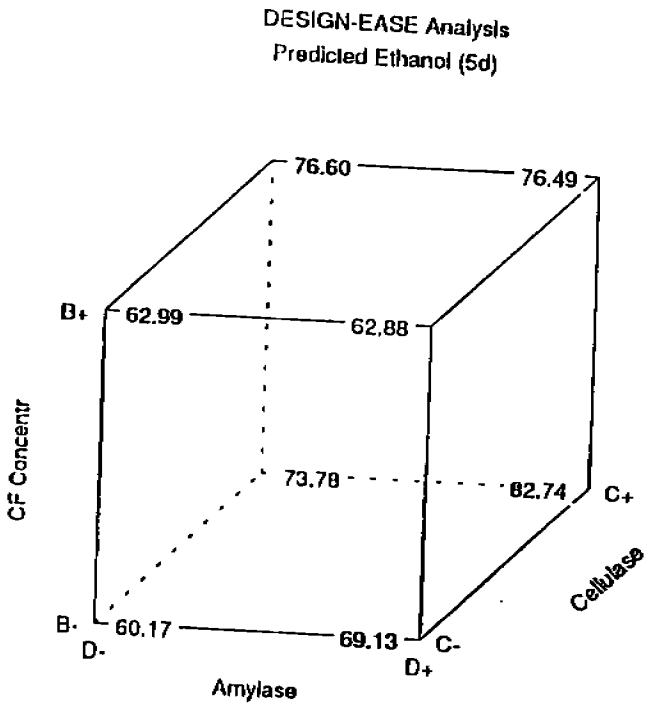
NREL

Figure 5

Ethanol Production (5 days)

Effects on Ethanol Yield





- CSL addition does not have an effect
- As opposed to 2-day data corn fiber concentration does not have any effect
- Cellulase concentration has a very strong posilive effect
- Glucoamylase concentration has a significant positive effect

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Appendix 1.A

In Model	Term	Coefficient	Standardized Effect	Sum of Squares
X	A:CSL Conc	-0.7320	-1.464	7.501
X	B:CF Concentr	-4.2283	-7.212	182.036
X	C:Cellulase	8.3194	17.788	1107.392
X	D:Amylase	1.6902	4.781	79.988
X	E:Temperature	1.5094	3.227	36.451
	AB	-0.2646	-0.179	0.112
	AC	0.3021	0.518	0.939
	AD	0.2653	0.313	0.344
	AE	-0.2571	-0.441	0.680
	BC	-1.0679	-0.969	3.284
X	BD	-1.3270	-2.103	15.486
	BE	-0.4321	-0.392	0.538
	CD	-0.3304	-0.474	0.786
	CE	0.8419	1.114	4.344
X	DE	1.0729	1.539	8.288

Analysis of Ethanol (2d)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	PROB > F
MODEL	1386.2311	7	198.03	31.69	< 0.0001
RESIDUAL	62.4985	10	6.25		
*LACK OF FIT	51.4535	9	5.72	0.52	0.8020
*PURE ERROR	11.0450	1	11.04		
COR TOTAL	1448.7296	17			

ROOT MSE	2.5000	R-SQUARED	0.96
DEP MEAN	58.3872	ADJ R-SQUARED	0.93
C.V. %	4.2817	PRED R-SQUARED	0.86

Predicted Residual Sum of Squares (PRESS) = 204.41

* Residual = Lack-Of-Fit + Pure Error

FACTOR	COEFFICIENT ESTIMATE	DF	STANDARD ERROR	t FOR H0 COEFFICIENT=0	PROB > |t|
INTERCEPT	58.356424	1	1.005370		
A	-1.091830	1	0.683467	-1.60	0.1412
B	-2.428976	1	1.063756	-2.28	0.0455
C	8.656818	1	0.652783	13.26	< 0.0001
D	1.150380	1	0.519429	2.21	0.0512
E	0.834489	1	0.729834	1.14	0.2795
BD	-1.259530	1	0.503685	-2.50	0.0314
DE	0.674886	1	0.376885	1.79	0.1036

Final Equation in Terms of Coded Factors

Ethanol (2d) =

```
              58.3564
            - 1.0918 * A
            - 2.4290 * B
            + 8.6568 * C
            + 1.1504 * D
            + 0.8345 * E
            - 1.2595 * B * D
            + 0.6749 * D * E
```

Final Equation in Terms of Uncoded Factors

Ethanol (2d) =

```
              43.1360
            - 2.1837 * CSL Conc
            - 0.0585 * CF Concentr
            + 1.7314 * Cellulase
            - 0.1515 * Amylase
            + 0.0399 * Temperature
            - 0.0126 * CF Concentr * Amylase
            + 0.0337 * Amylase * Temperature
```

OBS ORD	ACTUAL VALUE	PREDICTED VALUE	RESIDUAL	LEVER	STUDENT RESID	COOK'S DIST	OUTLIER T VALUE	RUN ORD
1	48.71	50.97	-2.260	0.463	-1.234	0.164	-1.271	5
2	47.15	48.47	-1.317	0.475	-0.727	0.060	-0.709	3
3	49.44	46.74	2.696	0.424	1.422	0.186	1.510	17
4	45.60	47.58	-1.979	0.435	-1.053	0.107	-1.059	11
5	69.50	67.96	1.535	0.463	0.838	0.076	0.825	16
6	67.67	66.10	1.570	0.475	0.867	0.085	0.855	9
7	66.75	67.08	-0.326	0.424	-0.172	0.003	-0.163	2
8	60.47	61.87	-1.404	0.435	-0.747	0.054	-0.729	7
9	49.28	50.65	-1.371	0.372	-0.692	0.035	-0.673	4
10	60.77	61.13	-0.356	0.650	-0.241	0.013	-0.229	8
11	51.87	50.89	0.976	0.501	0.553	0.038	0.532	18
12	45.59	42.99	2.598	0.592	1.626	0.479	1.799	13
13	69.62	68.28	1.336	0.372	0.674	0.034	0.655	12
14	72.57	72.72	-0.151	0.650	-0.102	0.002	-0.097	6
15	58.89	62.49	-3.600	0.501	-2.038	0.521	-2.529	15
16	66.05	66.02	0.026	0.592	0.016	0.000	0.015	14
17	58.17	59.51	-1.337	0.088	-0.560	0.004	-0.540	10
18	62.87	59.51	3.363	0.088	1.408	0.024	1.492	1

Appendix I.C

In Model	Term	Coefficient	Standardized Effect	Sum of Squares
X	A:CSL Conc	0.3877	0.775	2.1041
X	B:CF Concentr	-3.3165	-5.657	111.9928
X	C:Cellulase	6.8044	14.548	740.7923
X	D:Amylase	2.4009	6.791	161.4000
X	E:Temperature	-0.4069	-0.870	2.6488
X	AB	5.7312	3.875	52.5556
X	AC	3.1792	5.450	103.9587
	AD	-1.9369	-2.287	18.3123
X	AE	2.1183	3.631	46.1555
	BC	-1.2346	-1.120	4.3897
X	BD	-3.2128	-5.093	90.7720
	BE	-0.2979	-0.270	0.2556
	CD	-0.3171	-0.455	0.7239
	CE	0.5419	0.717	1.7993
	DE	0.9421	1.351	6.3902

Analysis of Ethanol (5d)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	PROB > F
MODEL	1384.9602	9	153.88	12.66	0.0008
RESIDUAL	97.2584	8	12.16		
*LACK OF FIT	75.6760	7	10.81	0.50	0.7994
*PURE ERROR	21.5825	1	21.58		
COR TOTAL	1482.2186	17			

ROOT MSE	3.4867	R-SQUARED	0.93
DEP MEAN	70.7933	ADJ R-SQUARED	0.86
C.V. %	4.9252	PRED R-SQUARED	0.71

Predicted Residual Sum of Squares (PRESS) = 434.74

* Residual = Lack-Of-Fit + Pure Error

FACTOR	COEFFICIENT ESTIMATE	DF	STANDARD ERROR	t FOR H0 COEFFICIENT=0	PROB > |t|
INTERCEPT	70.59697	1	1.40277		
A	-0.65236	1	0.97207	-0.67	0.5211
B	-0.85995	1	1.48377	-0.58	0.5782
C	6.80438	1	0.87168	7.81	< 0.0001
D	2.21264	1	0.75167	2.94	0.0186
E	-0.40687	1	0.87168	-0.47	0.6531
AB	2.74361	1	0.97207	2.82	0.0224
AC	3.02062	1	0.87168	3.47	0.0085
AE	2.58938	1	0.87168	2.97	0.0179
BD	-2.25832	1	0.72528	-3.13	0.0141

Final Equation in Terms of Coded Factors

Ethanol (5d) =

```
                70.59697
-            0.65236 * A
-            0.85995 * B
+            6.80438 * C
+            2.21264 * D
-            0.40687 * E
+            2.74361 * A * B
+            3.02062 * A * C
+            2.58938 * A * E
-            2.25832 * B * D
```

Final Equation in Terms of Uncoded Factors

Ethanol (5d) =

```
                90.94333
-           73.56522 * CSL Conc
-            0.05675 * CF Concentr
+            0.75675 * Cellulase
+            1.62081 * Amylase
-            0.74906 * Temperature
+            0.27438 * CSL Conc * CF Concentr
+            1.22525 * CSL Conc * Cellulase
+            1.29469 * CSL Conc * Temperature
-            0.00248 * CF Concentr * Amylase
```

OBS ORD	ACTUAL VALUE	PREDICTED VALUE	RESIDUAL	LEVER	STUDENT RESID	COOK'S DIST	OUTLIER T VALUE	RUN ORD
1	59.46	63.59	-4.132	0.494	-1.665	0.270	-1.927	5
2	51.29	51.57	-0.293	0.750	-0.162	0.008	-0.152	3
3	65.89	66.80	-0.913	0.599	-0.414	0.026	-0.391	17
4	64.70	64.13	0.570	0.599	0.258	0.010	0.243	11
5	79.93	77.15	2.778	0.494	1.120	0.122	1.140	16
6	75.87	75.59	0.282	0.750	0.162	0.008	0.152	9
7	65.65	68.38	-2.728	0.599	-1.236	0.229	-1.285	2
8	80.63	79.42	1.215	0.599	0.550	0.045	0.525	7
9	68.89	69.58	-0.694	0.494	-0.280	0.008	-0.263	4
10	73.72	73.86	-0.141	0.724	-0.077	0.002	-0.072	8
11	65.51	60.70	4.811	0.625	2.253	0.846	3.487	18
12	58.58	59.65	-1.074	0.625	-0.503	0.042	-0.478	13
13	72.28	71.16	1.121	0.494	0.452	0.020	0.428	12
14	69.36	69.15	-0.786	0.724	-0.430	0.048	-0.407	6
15	72.16	74.26	-2.099	0.625	-0.983	0.161	-0.981	15
16	82.03	83.67	-1.639	0.625	-0.768	0.098	-0.746	14
17	71.38	72.81	-1.430	0.090	-0.430	0.002	-0.407	10
18	77.95	72.81	5.140	0.090	1.545	0.024	1.726	1

	based on 3/22#'s 0mannose					CAT#95-034				Conditions		*based on	based on 2.54	
Run Order	2d Ethanol	5d Ethanol	7d Etoh Yi	CAT 7d	Residual Gluco	tl glucose oligo	tl galactose	tl xylose ol	tl arabinose	CSL	ECF conc	cellulase	amylase	temperatur
1	62.87	77.95	73.75		0					0.5	60	11	100	34
2	66.75	65.65	61.82	67.6	5	9.89	30.58	33.37	18.32	0	80	16	99	38
3	47.15	51.29	65.40		0					1	40	5	0	30
4	49.28	68.89	64.83	67.2	0	14.70	-1.67	31.69	6.19	0	40	5	0	30
5	48.71	59.46	62.32		0					0	40	5	0	38
6	72.57	88.36	74.36		0	6.27	-16.94	26.38	5.60	1	40	16	198	30
7	60.47	80.63	67.56	74.9	0	8.43	45.56	35.31	17.84	1	80	16	99	30
8	60.77	73.72	69.02	76.3	0	7.61	28.33	26.66	5.75	1	40	5	198	38
9	67.67	75.87	72.16		0					1	40	16	0	38
10	58.15	71.38	63.38		0					0.5	60	11	100	34
11	78.25	86.92	77.51		4					1	80	5	99	38
12	69.62	72.28	70.41	69.7	0	16.70	15.28	25.42	6.57	0	40	16	0	38
13	45.59	58.58	56.45	60.7	0	12.16	38.75	39.25	19.89	1	80	5	198	30
14	66.05	82.03	63.94	73.7	3	8.53	36.94	31.46	15.71	1	80	16	198	38
15	58.89	72.16	66.32		0					0	80	16	198	30
16	69.50	79.93	73.92		0					0	40	16	0	30
17	49.44	65.89	58.69		0					0	80	5	99	30
18	51.87	65.51	56.68		4					0	80	5	198	38

Appendix 1.1

Analysis of Glucose

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F VALUE	PROB > F
MODEL	42.83897	8	5.3549	14.57	0.0003
RESIDUAL	3.30868	9	0.3676		
*LACK OF FIT	3.30023	8	0.4125	48.82	0.1103
*PURE ERROR	0.00845	1	0.0085		
COR TOTAL	46.14765	17			

ROOT MSE	0.60633	R-SQUARED	0.93
DEP MEAN	0.99500	ADJ R-SQUARED	0.86
C.V. %	60.93728	PRED R-SQUARED	0.73

Predicted Residual Sum of Squares (PRESS) = 12.4870

* Residual = Lack-Of-Fit + Pure Error

FACTOR	COEFFICIENT ESTIMATE	DF	STANDARD ERROR	t FOR H0 COEFFICIENT=0	PROB > |t|
INTERCEPT	1.146938	1	0.192539		
A	-0.133306	1	0.196635	-0.68	0.5149
B	1.315161	1	0.273154	4.81	0.0010
C	-0.068750	1	0.151581	-0.45	0.6609
D	-0.235806	1	0.164855	-1.43	0.1864
E	0.942500	1	0.151581	6.22	0.0002
AB	-0.374113	1	0.272475	-1.37	0.2030
AD	0.188710	1	0.163349	1.16	0.2777
BE	0.870000	1	0.151581	5.74	0.0003

Final Equation in Terms of Coded Factors

Glucose =

1.14694
- 0.13331 * A
+ 1.31516 * B
- 0.06875 * C
- 0.23581 * D
+ 0.94250 * E
- 0.37411 * A * B
+ 0.18871 * A * D
+ 0.87000 * B * E

Final Equation in Terms of Uncoded Factors

Glucose =

10.94819
+ 1.60065 * CSL Conc
- 0.28529 * CF Concentr
- 0.01375 * Cellulase
- 0.08490 * Amylase
- 0.41687 * Temperature
- 0.03741 * CSL Conc * CF Concentr
+ 0.07548 * CSL Conc * Amylase
+ 0.01088 * CF Concentr * Temperature

OBS ORD	ACTUAL VALUE	PREDICTED VALUE	RESIDUAL	LEVER	STUDENT RESID	COOK'S DIST	OUTLIER T VALUE	RUN ORD
1	0.26	0.16	0.103	0.431	0.226	0.004	0.213	5
2	0.07	0.12	-0.046	0.624	-0.124	0.003	-0.117	3
3	0.35	0.80	-0.451	0.688	-1.331	0.433	-1.401	17
4	4.49	3.79	0.701	0.478	1.600	0.260	1.784	11
5	0.04	-0.13	0.166	0.431	0.362	0.011	0.344	16

6	0.14	0.12	0.017	0.624	0.045	0.000	0.042	9
7	4.74	4.29	0.451	0.688	1.331	0.433	1.401	2
8	0.22	0.03	0.194	0.478	0.442	0.020	0.421	7
9	0.05	0.01	0.038	0.431	0.084	0.001	0.079	4
10	0.26	0.07	0.187	0.635	0.512	0.051	0.490	8
11	3.60	3.58	0.023	0.661	0.064	0.001	0.061	18
12	0.35	0.07	0.280	0.483	0.643	0.043	0.621	13
13	0.13	0.02	0.111	0.431	0.242	0.005	0.229	12
14	0.05	-0.21	0.260	0.635	0.710	0.097	0.689	6
15	0.21	-0.19	0.395	0.661	1.119	0.271	1.137	15
16	2.80	3.56	-0.757	0.483	-1.737	0.313	-2.009	14
17	0.01	0.91	-0.901	0.070	-1.541	0.020	-1.693	10
18	0.14	0.91	-0.771	0.070	-1.319	0.015	-1.384	1

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-034 | Page 1 of 2

ject Title: 18 F.S. SSF Liquids (ET60)

NREL In-House ☐ | Current Subcontractor ☐ | CRADA ☒ | Other ☐

Name of Project Contact Person: Tammy K. Hayward	Date Work Completed: 3/06/95
NREL Notebook: 1561 p 21	Date Samples Delivered: 2/09/95
Samples from Feedstock Lot No.: N/A	Actual Hours Spent: 2
Summary of Requested Work: Analysis of liquor for monomeric and oligomeric sugars, acetic acid, HMF, fufural, and total dissolved solids.	Proposed Approach: Standard LAPs by validated outside laboratory.

Work Required: Sample Prep ☒ | Acid Digest ☒ | HPLC ☒ | YSI ☐ | GC ☒ | Other:

Results and Comments ☐ % As Received ☐ % Dry Weight ☒ mg/mL ☐ Other:

	Sample		G	X	GA	A	M	SA	LA	GLY	AC	HMF	FL	ET
1	F.S. SSF #8. 95-034-69, as received	ave	0.81	10.25	2.96	8.17	7.84	0.22	2.88	0.74	2.12	0.00	0.03	15.83
		sd	0.00	0.06	0.01	0.03	0.00	0.03	0.04	0.01	0.01	0.00	0.00	---
	- following 4% acid hydrolysis	ave	3.13	16.03	3.98	8.94	7.53	n/a	n/a	n/a	n/a	n/a	n/a	n/a
		sd	---	---	---	---	---	---	---	---	---	---	---	---
2	F.S. SSF #7. 95-034-70. as received	ave	1.27	19.42	4.37	14.24	14.60	1.85	3.46	1.27	3.93	0.00	0.03	28.9
		sd	---	---	---	---	---	0.04	0.07	0.02	0.06	0.00	0.00	---
	- following 4% acid hydrolysis	ave	6.41	34.73	8.15	19.02	16.83	n/a	n/a	n/a	n/a	n/a	n/a	n/a
		sd	---	---	---	---	---	---	---	---	---	---	---	---
3	F.S. SSF #4. 95-034-71. as received	ave	0.12	10.16	1.56	8.09	6.94	0.00	0.95	0.53	1.93	0.00	0.03	13.15
		sd	0.00	0.00	0.00	0.02	0.02	0.01	0.02	0.00	0.01	0.00	0.00	---
	- following 4% acid hydrolysis	ave	4.60	17.03	1.50	8.92	6.60	n/a	n/a	n/a	n/a	n/a	n/a	n/a
		sd	---	---	---	---	---	---	---	---	---	---	---	---

A=arabinose; AC=acetic acid; AT=total ash; ET=ethanol; FL=furfural; G=glucose; GA=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LA=lactic acid; LAS=acid soluble lignin; LKL=Klason lignin; M=mannose; nd=not detected; nr=not requested; P=protein; SA=succinic acid; ST=starch; TS=total solids; X=xylose

Name(s) of CAT Staff Working on Project: Larry Brown, David Templeton

Larry Brown
David Templeton

Reviewed by: Tina Ehrman

Tina Ehrman

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-034 | Page 2

Results and Comments ☐ % As Received ☐ % Dry Weight ☒ Other: MG/ML

Sample		G	X	GA	A	M	SA	LA	GLY	AC	HMF	FL	ET
4 F.S. SSF #6, 95-034-72, as received	ave	0.77	11.89	2.13	8.41	8.49	0.81	2.56	0.68	1.93	0.00	0.03	16.0
	sd	0.00	0.00	0.00	0.02	0.02	0.01	0.02	0.00	0.01	0.00	0.00	---
- following 4% acid hydrolysis	ave	2.68	17.61	1.52	9.16	8.24	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	sd	---	---	---	---	---	---	---	---	---	---	---	---
5 F.S. SSF #12, 95-034-73, as received	ave	0.15	10.73	3.26	8.15	7.35	0.17	0.90	0.61	1.89	0.00	0.03	13.70
	sd	0.00	0.01	0.00	0.02	0.03	0.03	0.00	0.00	0.00	0.00	0.00	---
- following 4% acid hydrolysis	ave	5.24	16.24	3.81	9.03	7.24	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	sd	---	---	---	---	---	---	---	---	---	---	---	---
6 F.S. SSF #14, 95-034-74, as received	ave	3.17	20.63	5.43	14.80	15.00	2.04	2.77	1.19	3.67	0.00	0.04	29.3
	sd	---	---	---	---	---	0.03	0.05	0.01	0.04	0.00	0.00	---
- following 4% acid hydrolysis	ave	8.37	34.27	8.09	19.01	16.76	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	sd	---	---	---	---	---	---	---	---	---	---	---	---
F.S. SSF #2, 95-034-75, as received	ave	5.29	21.37	5.54	13.26	13.99	1.98	1.22	0.92	3.63	0.00	0.04	26.1
	sd	---	---	---	---	---	0.03	0.03	0.02	0.05	0.00	0.00	---
- following 4% acid hydrolysis	ave	11.32	36.04	8.39	20.17	15.85	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	sd	---	---	---	---	---	---	---	---	---	---	---	---
8 F.S. SSF #13, 95-034-76, as received	ave	0.27	19.59	5.75	15.55	11.64	0.45	2.78	0.83	3.32	0.00	0.03	24.2
	sd	0.01	0.02	0.03	0.03	0.08	0.02	0.27	0.01	0.05	0.00	0.00	---
- following 4% acid hydrolysis	ave	7.68	36.61	8.54	20.88	13.99	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	sd	---	---	---	---	---	---	---	---	---	---	---	---

A=arabinose; AC=acetic acid: AT=total ash; ET=ethanol; FL=furfural: G=glucose: GA=galactose: GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LA=lactic acid: LAS=acid soluble lignin: LKL=Klason lignin: M=mannose; nd=not detected: nr=not requested; P=protein: SA=succinic acid: ST=starch; TS=total solids; X=xylose

Std	Dsn Id	Run	Block	CSL Conc % (v/w) Factor	CF Concentr wt% Factor	Cellulase IU/g cellul Factor	Amylase IU/g oligom Factor	Temperature degrees C Factor	Ethanol (2d) % (GGM) Response	Ethanol (5d) % theor Response	Ethanol (7d) % theor Response	Glucose g/L Response
1	1	5	1	0.00	40.00	5.00	0.00	38.00	48.71	59.46	62.32	0.26
2	2	3	1	1.00	40.00	5.00	0.00	30.00	47.15	51.29	65.40	0.07
3	3	17	1	0.00	80.00	5.00	10.00	30.00	49.44	65.89	58.69	0.35
4	4	11	1	1.00	80.00	5.00	10.00	38.00	45.60	64.70	57.70	4.49
5	5	16	1	0.00	40.00	15.00	0.00	30.00	69.50	79.93	73.92	0.04
6	6	9	1	1.00	40.00	15.00	0.00	38.00	67.67	75.87	72.16	0.14
7	7	2	1	0.00	80.00	15.00	10.00	38.00	66.75	65.65	61.82	4.74
8	8	7	1	1.00	80.00	15.00	10.00	30.00	60.47	80.63	67.56	0.22
9	9	4	1	0.00	40.00	5.00	0.00	30.00	49.28	68.89	64.83	0.05
10	10	8	1	1.00	40.00	5.00	20.00	38.00	60.77	73.72	69.02	0.26
11	11	18	1	0.00	80.00	5.00	20.00	38.00	51.87	65.51	56.68	3.60
12	12	13	1	1.00	80.00	5.00	20.00	30.00	45.59	58.58	56.45	0.35
13	13	12	1	0.00	40.00	15.00	0.00	38.00	69.62	72.28	70.41	0.13
14	14	6	1	1.00	40.00	15.00	20.00	30.00	72.57	88.36	74.36	0.05
15	15	15	1	0.00	80.00	15.00	20.00	30.00	58.89	72.16	66.32	0.21
16	16	14	1	1.00	80.00	15.00	20.00	38.00	66.05	82.03	63.94	2.80
17	0	10	1	0.50	60.00	10.00	10.00	34.00	58.17	71.38	63.38	0.01
18	0	1	1	0.50	60.00	10.00	10.00	34.00	62.87	77.95	73.75	0.14